Upper Keratinocytes of Psoriatic Skin Lesions Express High Levels of NAP-1/IL-8 mRNA In Situ

Reinhard Gillitzer, Rudolf Berger, Volker Mielke, Christoph Müller, Klaus Wolff, and Georg Stingl

Department of Dermatology I (RG, RB, KW, GS), Division of Cutaneous Immunobiology, University of Vienna Medical School, Vienna, Austria; Department of Pathology (CM), University of Bern, Bern, Switzerland; and Department of Dermatology (VM), University of Kiel, Kiel, F.R.G.

In order to better understand the factors regulating disease promotion and activity in psoriasis (PS), we searched for the in situ expression of mRNA for various cytokines in long-standing PS skin lesions. Specific hybridization with a NAP-1/IL-8 anti-sense RNA probe was keratinocyte associated and yielded strong and specific signals exclusively in the upper layers of the lesional epidermis, but not in uninvolved skin from psoriatic patients or normal skin from non-psoriatics. Interestingly, NAP-1/IL-8 transcripts were focally clustered in a spotty pattern predominantly between the tips of elongated papillae, but were absent in the lower epidermal region and the dermal compartment. We consistently failed to detect appreciable numbers of TNF-α and/or IL-6 mRNA-containing cells in psoriatic lesions. These results support the notion that IL-8, rather than IL-6, is an important disease-promoting cytokine in PS. In view of the known in vitro and in vivo effects of IL-8, it is conceivable that this substance greatly contributes to the major pathologic changes seen in psoriatic skin, i.e., keratinocyte hyperproliferation and leucocyte infiltration. In this case, local pharmacologic down-regulation of NAP-1/IL-8 activity could be a promising therapeutic strategy in PS. *J Invest Dermatol 97:73–79, 1991*

Cytokines are thought to play an important role in the pathogenesis of various dermatoses. In this regard, particular attention has focused on psoriasis (PS) as a prototype of an inflammatory skin disease with keratinocyte (KC) hyperproliferation and pronounced leukocytic infiltration. In this disease, abnormal levels of TNF-α/β [1], TGF-α [2], IL-1 [1,3,4,5], and IL-6 [6] have been reported and, consequently, linked to its pathogenesis.

Most recently, on the basis of biochemical analysis and in vitro studies, it became evident that NAP-1/IL-8 may very well be one of the hallmark cytokines in PS lesions. High amounts of NAP-1/IL-8 [1,7,8,9] have been found in scales from PS lesions, but not in uninvolved psoriatic and non-psoriatic skin. Although these data support the role of NAP-1/IL-8 as a PS-promoting factor, it is not known at present whether the elevated levels of this cytokine in psoriatic scales are of KC origin [10], or alternatively, predominantly derived from other cells within psoriatic skin. The latter possibility has to be entertained in view of previous findings showing that, upon appropriate stimulation, fibroblasts [10,11], lymphocytes [12], endothelial cells (EC) [13], and monocytes produce high levels of NAP-1/IL-8 (reviewed in [14]). In order to address this issue, we employed an in situ hybridization technique allowing the detection and microanatomic localization of mRNA expression of various cytokines.

Manuscript received December 19, 1990; accepted for publication January 11, 1991.

This work was supported by grants from the Deutsche Forschungsgemeinschaft, Federal Republic of Germany and from the Jubiläumsfonds der Österreichischen Nationalbank, Vienna, Austria.

This work was presented in part at the VXIIIth meeting of the Arbeitsgemeinschaft Dermatologische Forschung ADF, Mannheim, FRG, November 9–11, 1990.

Reprint requests to: Reinhard Gillitzer, Department of Dermatology, University of Würzburg Medical School, Josef-Schneider-Strasse 2, D-8700 Würzburg, FRG.

Abbreviations:
EC: endothelial cells
ELAM-1: endothelial leucocyte adhesion molecule 1
ICAM-1: intercellular adhesion molecule 1
IL-1: interleukin 1
IL-6: interleukin 6
KC: keratinocytes
NAP-1/IL-8: neutrophil-activating peptide 1/interleukin 8
PS: psoriasis
TGF: transforming growth factor
TNF: tumor necrosis factor

MATERIALS AND METHODS

Selection and Preparation of Skin Sections Fresh incisional biopsy specimens were taken from uninvolved (n = 5) and involved (n = 8) skin of patients with untreated, long-standing PS and, for control purposes, from seven healthy volunteers. The material was snap-frozen in OCT-compound (Tissue-Tek, Miles Scientific, Naperville, IL) using isopentane pre-cooled in liquid nitrogen and stored at −70°C until use. Subsequently, 4-μm cryostat sections were prepared and used for either immunohistologic or in situ hybridization purposes.

Immunohistology We studied selected features of psoriatic skin lesions by using the following monoclonal antibodies (MoAb): MoAb anti-CD3 (Leu-4, 1:400) reacting with the T-cell receptor-associated CD3 antigen, and anti-CD45 (HLe, 1:300) specific for a cell membrane moiety on all human leukocytes, also termed leucocyte common antigen (LCA) were purchased from Becton-Dickinson, Sunnyvale, CA. MoAb anti-ICAM-1 (1:100) reacting with the intercellular-adhesion molecule-1 (ICAM-1/CD54) [15] and MoAb anti-ELAM-1 (1:100) reacting with the endothelial leucocyte adhesion molecule-1 (ELAM-1) [16] were a generous gift from Dr. D. O. Haskard, Department of Rheumatology, U.M.D.S., London, U.K. ICAM-1 mediates a wide range of leukocyte interac-

0022-202X/91/$03.50

tions with other cells (e.g., EC or KC), whereas ELAM-1 is restricted to EC and mediates neutrophil adhesion. EN-4, a MoAb reacting with all EC of mature blood and lymphatic vessels (pan-endothelial cell MoAb, 1:1000) was obtained from Sera-Lab, Essex, U.K.

Single Labeling: For single-color staining a three-step streptavidin-biotin alkaline phosphatase immunohistologic procedure was used as described elsewhere [17,18]. Briefly, after blocking Fc-receptors with 20% sheep serum in phosphate-buffered saline (PBS), sections were overlaid with the specific MoAb followed by biotin-conjugated sheep anti-mouse Ig (Amersham, Amersham, UK). Finally, after thorough washing steps, sections were incubated with preformed streptavidin-biotin alkaline phosphatase complex (streptABComplex-AP, Dakopatts, Copenhagen, Denmark). Labeling was visualized with the substrates naphthol AS-MX phosphate (NAMP)/Fast Red or bromochloro-indolyl-phosphate (BCIP)/nitroblue tetrazolium (NBT), respectively.

Control: For control purposes, the first MoAb was either omitted or replaced by an irrelevant isotype-matched reagent. These experiments consistently yielded negative results.

In Situ Hybridization

Preparation of ^{35}S-Labeled mRNA Probes: TNF-α cDNA was obtained from Genentech Inc., San Francisco, CA [19] and subcloned in pGEM-1 as previously described [11]; IL-6 cDNA subcloned in pGEM-4 was a generous gift from Dr. T. Hirano and Dr. T. Kishimoto, University of Osaka, Osaka, Japan [20] and NAP-1/IL-8 (3-10c) cDNA was obtained from Dr. C. Weissmann, University of Zürich, Switzerland [21] and subcloned in pGEM-1/2 as previously described [11]. Riboprobes were prepared from these cDNA according to the method of Mueller et al [22]. Briefly, after linearization of plasmid DNA with appropriate restriction enzymes, ^{35}S-labeled RNA sense and anti-sense stranded probes were obtained by in vitro transcription with SP6 and T7 RNA-polymerases, respectively. The template DNA was removed by DNAse (Pharmacia LKB Biotechnology, Uppsala, Sweden) treatment. Seventy to ninety-five percent of the ^{35}S-UTP nucleotides were incorporated into the riboprobes, which were subsequently reduced to an average length of 100–200 base pairs by alkaline hydrolysis. After multiple ethanol precipitations the riboprobe was resuspended at 2×10^6 cpm/μl in a buffer (pH 7.5) consisting of 10 mM Tris-HCl and 1 mM EDTA.

Hybridization Procedure: The protocol described by Mueller et al [22] was used with minor alterations. Briefly, paraformaldehyde-fixed cryostat sections were incubated with 1 μg/ml proteinase K (Boehringer, Mannheim, FRG) for 30 min at 37°C. This procedure facilitates the accessibility of radioactive probes to cellular mRNA [23–25]. Sections were then refixed in 4% paraformaldehyde. After acetylation with acetic anhydride in 0.1 M triethanolamine (pH 8.0, for 10 min), slides were dehydrated by incubation in graded alcohol solutions and then air dried. Afterwards, sections were overlaid with 20 μl of the hybridization solution consisting of 50% formamide, 300 mM NaCl, 20 mM Tris-HCl, 5 mM EDTA (pH 8.0), 1 × Denhardt's solution, 10% dextran sulfate, 100 mM dithiothreitol, as well as the heat-denatured probe (final concentration: 2×10^5 cpm/μl). The slides were mounted with coverslips, sealed, and hybridized at 46–47°C for 12–16 h. Every anti-sense and, for control purposes, every sense probe was hybridized with at least three sections of the same biopsy. (As an additional control, slides were treated with 10 μg/ml RNase [Boehringer] in 2 × sodium saline citrate [SSC] for 30 min at 37°C prior to hybridzation.) Non-hybridized probes were removed by several high-stringency washing procedures with 50% formamide solution containing 2 × SSC and 5 mM EDTA at 54–57°C. To minimize non-specific background, non-complementary unbound single-stranded probe-RNA was digested with RNase A (20μl/ml) and RNase T1 (1U/ml) (Boehringer, Mannheim, FRG) for 30 min at 37°C. For autoradiography, slides were dipped in a NTB-2 Kodak solution (1:2 in

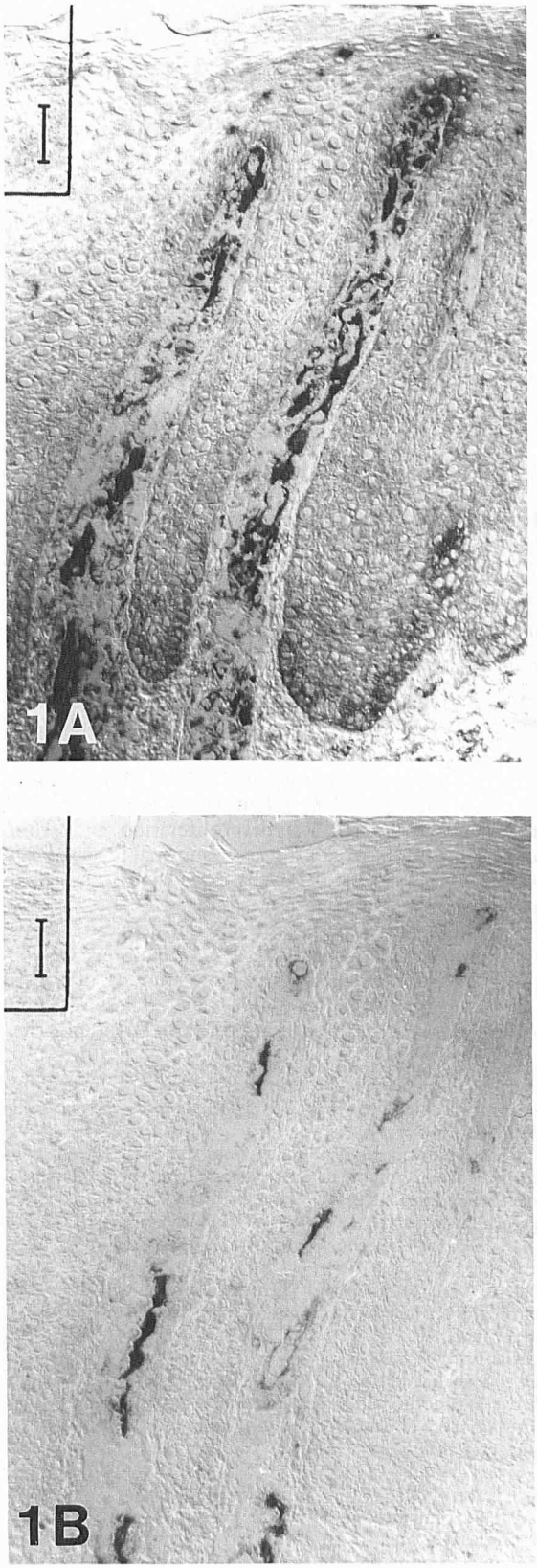
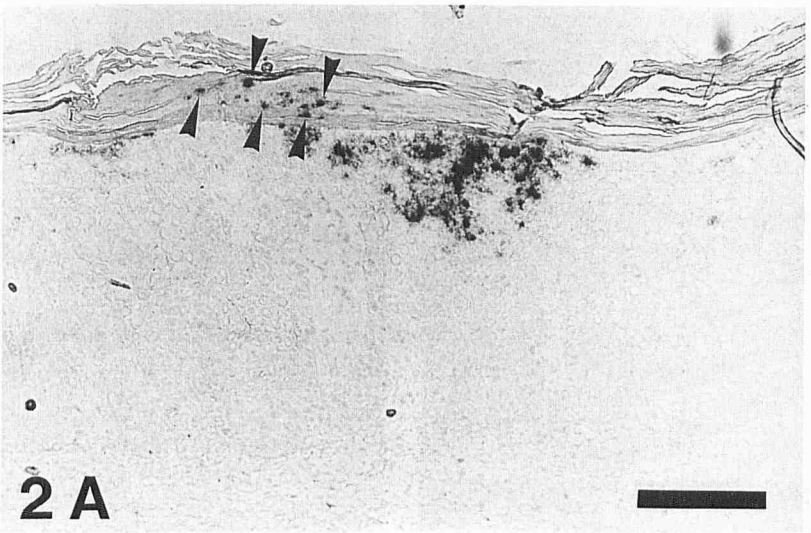
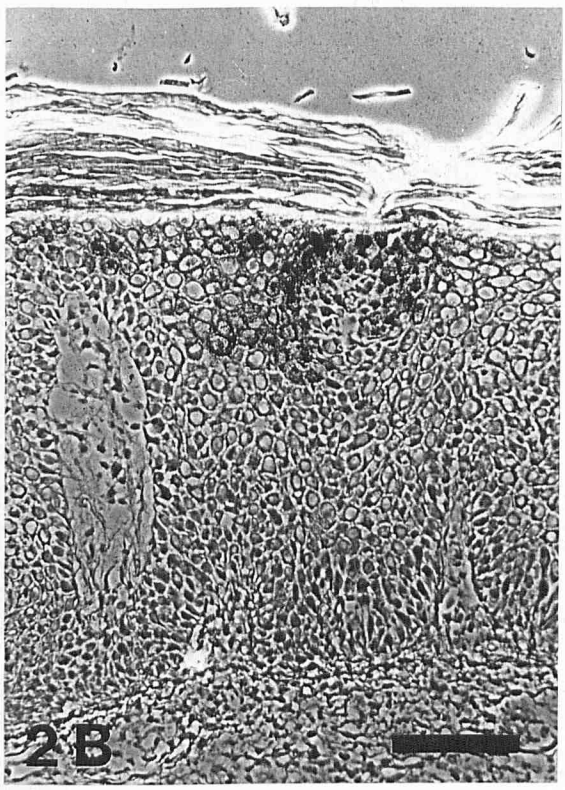


Figure 1. *A*, Immunohistological detection of ICAM-1 expression in a lesion of chronic plaque psoriasis. Endothelial cells of elongated capillary vessels exhibit strong anti-ICAM-1 reactivity; in addition certain infiltrating leukocytes as well as some basal keratinocytes are positively labeled. *B*, ELAM-1 expression in a serial section. Labeling is restricted to EC and, in general, less pronounced than anti-ICAM-1 staining. (Three-step streptavidin-biotin-alkaline phosphatase method and use of interference-contrast microscopy; *bar*, 50 μm, ×20 objective.)





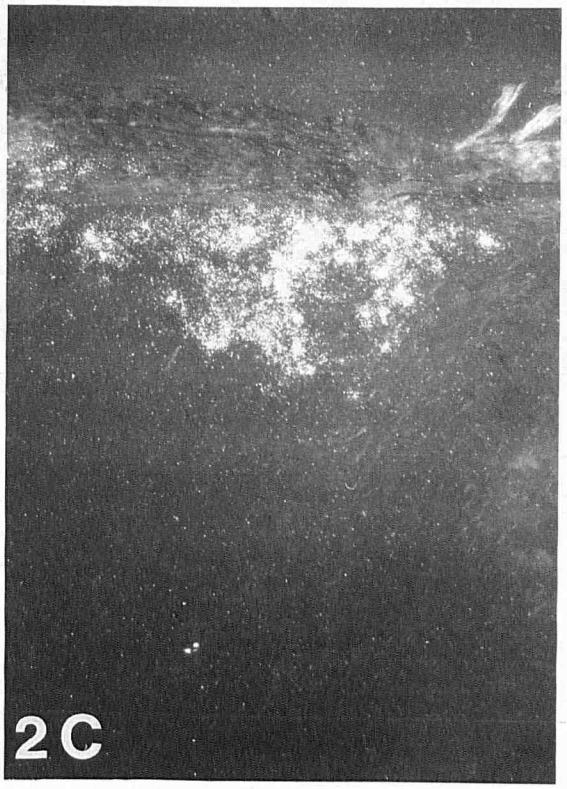
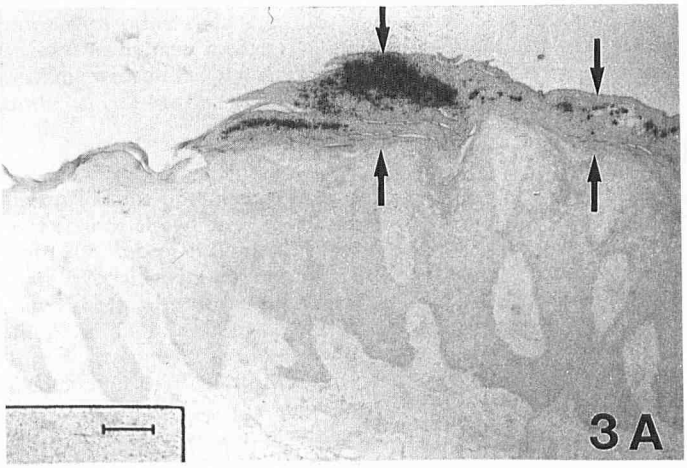


Figure 2. In situ hybridization for NAP-1/IL-8 mRNA in psoriatic lesions using ^{35}S-labeled RNA anti-sense probe. Clustering of silver grains is indicative of cells containing NAP-1/IL-8 mRNA transcripts. *A*, Most NAP-1/IL-8 mRNA$^+$ cells are focally arranged in the upper stratum malpighii with few positive cells in the parakeratotic layer (*arrowhead*), (*bar*, 200 μm, ×10). *B*, Detail of *A* (*bar*, 100 μm, ×20 objective). *C*, Enhancement of silver grains with epipolarization illumination (×20).

800 mM ammonium acetate), air dried, and exposed for 1–5 weeks until developed.

RESULTS

Immunohistology In addition to the mixed (CD45$^+$ cells >> CD3$^+$ cells) inflammatory infiltrate that was predominantly localized in the papillary dermis, sections of lesional psoriatic skin exhibited the typical epidermal acanthosis, parakeratotic hyperkeratosis, hypogranulosis, and dermal changes (elongated papillary vessels) seen in long-standing plaque PS. Analysis of the expression pattern of adhesion molecules ICAM-1 and ELAM-1 revealed striking differences between lesions of PS and uninvolved skin from psoriatics or normal skin. Whereas EC of large dermal vessels of both the superficial and deep vascular plexus displayed strong anti-ICAM-1 reactivity in lesions of PS as well as in uninvolved/normal skin, EC lining papillary capillaries as evidenced by sequential staining with MoAb EN-4 exhibited pronounced anti-ICAM-1 immunolabeling only in lesional psoriatic skin (Fig 1*A*). In agreement with previous reports [30], we identified a considerable number of ICAM-1$^+$ infiltrating leukocytes as well as some ICAM-1-bearing basal KC. Anti-ELAM-1/EN-4 immunolabeling of serial sections revealed that EC of capillaries within uninvolved psoriatic and normal skin are ELAM-1 negative. In contrast, anti-ELAM-1-reactive EC were frequently found in papillary capillaries of psoriatic lesions. It appeared that, within a given vessel, ELAM-1 and ICAM-1 expression correlate with each other (Fig 1*A,B*). However, the intensity of



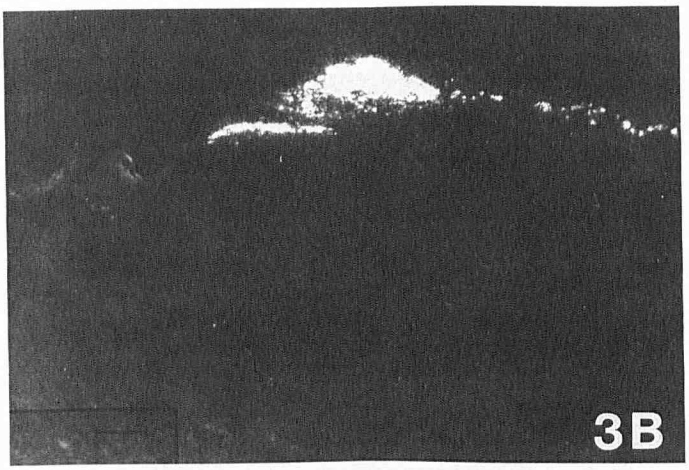


Figure 3. In situ hybridization for NAP-1/IL-8 mRNA in psoriatic lesion. NAP-1/IL-8 mRNA$^+$ keratinocytes are exclusively found in the parakeratotic layer as indicated (*arrows*). *A*, Bright field; *B*, signal enhancement with epipolarization illumination (*bar*, 50 μm, $\times$16.8).

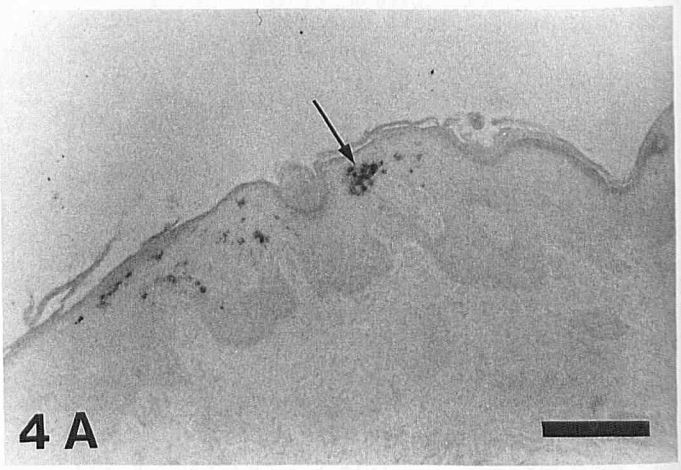
anti-ELAM-1 labeling was always lower than that of anti-ICAM-1 labeling.

In Situ Hybridization The most conspicuous finding of this study was the detection of highly abundant hybridization signals with the NAP-1/IL-8 anti-sense probe, but not with the NAP-1/IL-8 sense probe in the lesional, acanthotic psoriatic epidermis in 7 of 8 patients investigated (Figs 2–4). No hybridization occurred when sections were predigested with RNase. Typically, NAP-1/IL-8 transcripts were present as focal clusters in the upper malpighian layer (Fig 2) and/or the parakeratotic stratum corneum (Fig 3), but were entirely absent in the lower epidermal layers. The single silver grain foci covered an area of 5–100 cells (Figs 2–4). The distances between these individual pockets of NAP-1/IL-8 mRNA expression were variable but occasionally exceeded the length of 1 mm. By using phase or interference contrast microscopy to better visualize the epidermal structure, the hybridization signal was found to be clearly associated with upper-level KC (Fig 2). However, the possibility that a few scattered neutrophils in the upper layer also contain NAP-1/IL-8 mRNA cannot be definitively excluded. In sharp contrast to the upper psoriatic epidermis, the dermis of psoriatic lesions was completely devoid of any NAP-1/IL-8 message detectable in situ, as were all biopsy specimens from uninvolved psoriatic and non-psoriatic normal skin. Staining for CD3$^+$/CD45$^+$ cells on adjacent sections of hybridized tissue revealed a frequent, but not regular association between the location of NAP-1/IL-8 mRNA expression and the location of leukocytes (CD45$^+$ cells >> CD3$^+$ cells) infiltrating the epidermis (Fig 4*A*,*B*).

In contrast to the abundant presence of NAP-1/IL-8 mRNA, only few scattered cells within the lesional psoriatic epidermis gave a specific hybridization signal with the TNF-α anti-sense probe (data not shown). IL-6 mRNA, while undetectable in the entire epidermis, was specifically expressed in a small number of cells in the dermis (Fig 5). A similar picture was obtained when cryostat sections of uninvolved skin from psoriatics or normal non-psoriatic skin were hybridized with the IL-6 probe (not shown).

DISCUSSION

Whereas the ultimate cause of PS is yet unknown, several factors have been implicated in governing disease activity including arachidonic acid derivates (e.g., leukotriene B4 [LTB4] and 12-hydroxyeicosatetraenoic acid [12-HETE]), C5a, and platelet-activating factor (PAF) (summarized in [26]). Recently, particular attention has focused on the role of cytokines in the pathogenesis of the disease and there now exist several reports on the altered expression of the cytokines IL-1 [1,3–5], IL-6 [6], NAP-1/IL-8 [7–9], and TGF-α [2] in extracts of psoriatic skin. These studies provide useful infor-



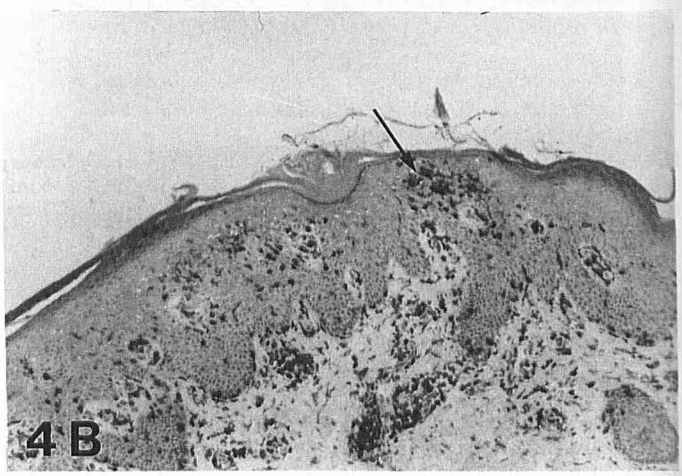


Figure 4. *A*, In situ hybridization for NAP-1/IL-8 mRNA in a psoriatic lesion. *B*, A serial section is stained for CD45$^+$ cells (3-step-streptavidin alkaline phosphatase method). A cluster of CD45$^+$ cells is localized at the corresponding site of high NAP-1/IL-8 message as indicated (*arrow*) (*bar*, 200 μm, magnification $\times$10).

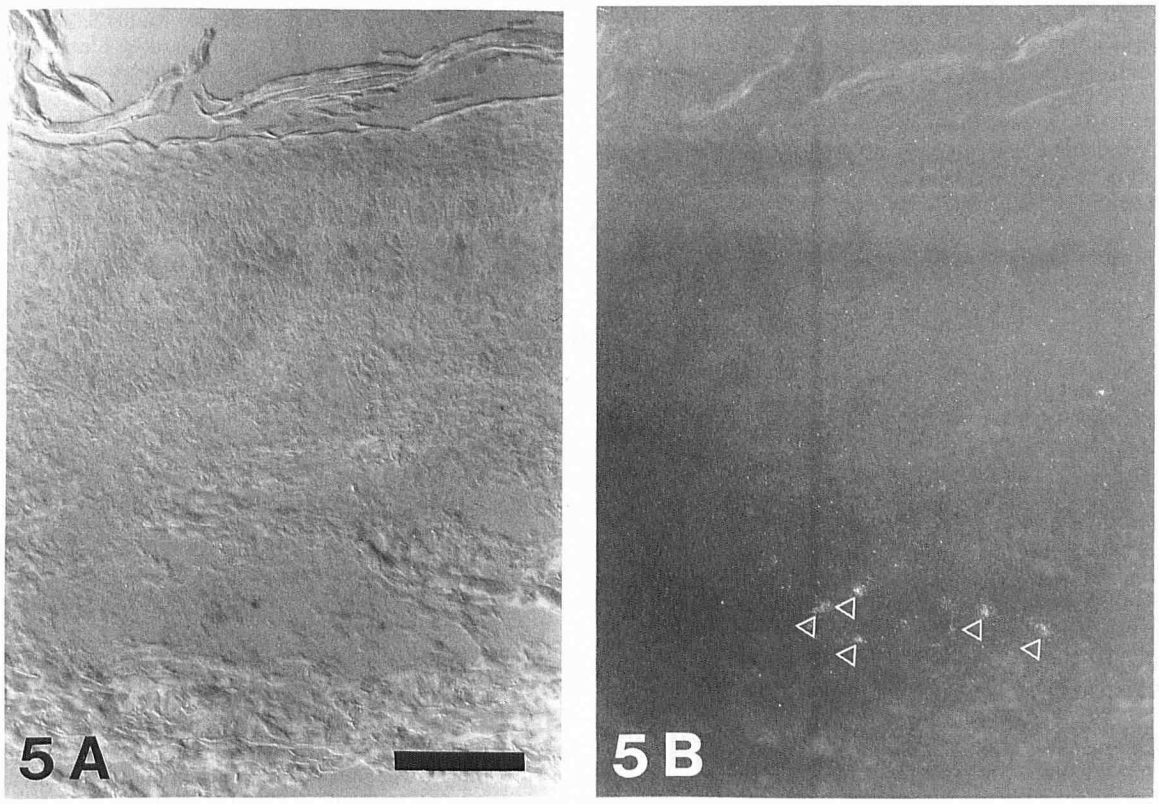


Figure 5. In situ hybridization for IL-6 mRNA in a psoriatic lesion. As opposed to a few IL-6 mRNA-expressing cells in the dermis (*white arrowheads*), the epidermis is devoid of any specific IL-6 hybridization signals. *A*, Bright field; *B*, epipolarization illumination (*bar*, 100 μm, $\times$20).

mation about the biochemical properties and the bioactivity of the various factors; however, they do not allow valid conclusions about the actual source and the microanatomic site of cytokine production. We therefore decided to study the expression of various cytokine genes (IL-6, NAP-1/IL-8, TNF-α) by in situ hybridization and found foci of abundant NAP-1/IL-8 transcripts in the upper layers of the lesional psoriatic epidermis, but not in either uninvolved skin from psoriatics or in normal skin of healthy volunteers. Clearly, upper-level KC are the major, if not the only, source of NAP-1/IL-8 mRNA within psoriatic lesions. In contrast to previous speculations [1,27], mononuclear cells infiltrating psoriatic lesions as well as EC and fibroblasts were uniformly quiescent. This is somewhat surprising because, at least in vitro, all these cell types can be induced to produce abundant NAP-1/IL-8 mRNA and protein [10–14,28]. Nevertheless, the mere possibility that our in situ technique detects message in KC, but misses message in other cells can be excluded by its successful application for the detection of NAP-1/IL-8 mRNA in the dermal compartment of various other dermatoses (Gillitzer et al, in preparation). Our finding of NAP-1/IL-8 mRNA in the upper layers of lesional psoriatic skin is in agreement with the reported identification of NAP-1/IL-8 protein in psoriatic scales [7–9] and NAP-1/IL-8 mRNA in the epidermis of psoriatic lesions [29]. Thus, we may conclude that NAP-1/IL-8 mRNA within the psoriatic epidermis is translated into functional ,protein. Our finding that abundant in situ signals were not restricted to the upper malphighian layers, but were also found in the parakeratotic horny layer (where novel RNA and protein synthesis is unlikely to occur), possibly mirrors a long half time of NAP-1/IL-8 mRNA corresponding to the transition time of KC from the granular into the parakeratotic compartment. In contrast to our finding concerning the spatial distribution of NAP-1/IL-8, other investigators, using immunohistologic approaches, have come to different conclusions. Barker et al [29] localized NAP-1/IL-8 in basal keratinocytes; Sticherling et al [37] reported that NAP-1/IL-8-immunoreactivity was confined to suprabasal or all KC depending on the MoAb used. The NAP-1/IL-8-immunoreactivity in psoriatic epidermis was inversely correlated with the inflammatory tissue reaction. Further studies are needed to clarify whether differences in methodology (in situ hybridization versus immunocytochemistry) can account for the discrepancy in the results.

In view of the peculiar topobiology of NAP-1/IL-8 gene expression and the frequent detection of infiltrating leukocytes in the close vicinity of NAP-1/IL-8 gene expression, we propose the following concept for the inflammatory component of the psoriatic lesion: Similar to what has been reported for LTB4 and C5a [26], an ascending NAP-1/IL-8 concentration gradient is formed within psoriatic skin. This results in the attraction of neutrophils (C5a, LTB4, NAP-1/IL-8; [26]) and lymphocytes (NAP-1/IL-8; [38]) into the psoriatic epidermis and, occasionally, leads to the formation of Munro's microabscesses. This process is probably facilitated by the increased extravasation of inflammatory cells through ICAM-1 and ELAM-1–expressing EC of the papillary microvessels. Thus, our data highlight the dual principle of adhesion molecule expression in conjunction with chemotactic gradient formation, as proposed and indirectly demonstrated by others [28,29]. However, even the chemotactic activity of NAP-1/IL-8 for lymphocytes has been shown in vitro [38]; our observation of regular colocalization of NAP-1/IL-8 mRNA in association with $CD45^+$ cells ($CD45^+$ cells >> $CD3^+$ cells) favors the preferential activity of NAP-1/IL-8 on $CD45^+CD3-$ cells.

The very recent finding that NAP-1/IL-8 is a potent mitogen for human keratinocytes in vitro [31] suggests that, in psoriasis, NAP-1/IL-8 derived from upper KC acts in a paracrine fashion as a growth-promoting factor for KC of the lower epidermal compartment. Thus, two major pathologic features of PS, i.e., epidermal hyperplasia and inflammation, can be explained through NAP-1/IL-8 as a central driving molecule. This by no means excludes the possibility that other substances play a role in stimulating KC prolif-

eration in PS. Whereas we (this study) and others [1,36] were not able to confirm the previous finding of elevated IL-6 mRNA and protein in lesions of PS [6], several laboratories have reported that TGF-α, a growth factor for KC, is overexpressed in psoriatic epidermis [2,32]. Similar to our finding with NAP-1/IL-8, Turbitt et al [32] have localized TGF-α mRNA to high-level KC rather than to basal KC in PS. These observations clearly document that, in PS, upper KC exhibit high metabolic activities. Delivery of mitogenic stimuli by these cells could account for the phenomenon that, whereas KC proliferation in normal human epidermis is restricted to the basal layer, proliferation and mitotic figures in psoriatic epidermis can be readily identified in both basal and suprabasal KC (33–35).

At this moment, the factor(s) leading to NAP-1/IL-8 expression in lesions of PS is (are) unknown. The observations that, in lesional psoriatic skin, IL-1 activity is markedly reduced [4] and that TNF-α cannot be readily detected at either the mRNA level (this study) or at the functional level [1] indicate that these two cytokines do not play a major role in the induction of NAP-1/IL-8 transcription in psoriatic epidermis. On the other hand, it has recently been reported that the synergistic action of TNF-α and interferon-γ stimulate NAP-1/IL-8 expression by cultured human KC [28]. The relevance of this in vitro finding for the in vivo situation in psoriasis is presently unclear.

Although the role of NAP-1/IL-8 in the initiation of psoriatic lesions has yet to be determined by careful analysis of NAP-1/IL-8 gene expression in developing psoriatic papules, our data strongly suggest that NAP-1/IL-8 is an important disease-promoting cytokine. It may, therefore, be expected that interference with NAP-1/IL-8 expression would result in the regression, or even resolution, of psoriatic lesions. Our finding of abundant NAP-1/IL-8 mRNA in the upper layer of lesional psoriatic epidermis implies that a topical approach would be a most reasonable strategy to accomplish this goal.

REFERENCES

1. Gearing AJH, Fincham NJ, Bird CR, Wadhwa M, Cartwright AJE, Camp RDR: Cytokines in skin lesions of psoriasis. Cytokine 2:68–75, 1990
2. Elder JT, Fisher GJ, Lindquist PB, Bennet GL, Pittelkow MR, Coffey RJ, Ellingsworth L, Derynck R, Voorhees JJ: Overexpression of transforming growth factor α in psoriatic epidermis. Science 243:811–814, 1989
3. Hammerberg C, Fisher F, Baadsgaard O, Voorhees JJ, Cooper KD: Characterization of IL-1 and inhibitor in psoriatic and normal skin (abstr). J Invest Dermatol 92:439, 1989
4. Cooper KD, Hammerberg C, Baadsgaard O, Elder JT, Chan LS, Sauder DN, Voorhees JJ, Fisher G: IL-1 activity is reduced in psoriatic skin. Decreased IL-1α and increased nonfunctional IL-1β. J Immunol 144:4593–4603, 1990
5. Ristow H-H: A major factor contributing to epidermal proliferation in inflammatory skin diseases appears to be interleukin 1 or a related protein. Proc Natl Acad Sci USA 84:1990–1994, 1987
6. Grossman RM, Krueger J, Yourish D, Granelli-Piperno A, Murphy DP, May LT, Kupper TS, Sehgal PB, Gottlieb AB: Interleukin 6 is expressed in high levels in psoriatic skin and stimulates proliferation of cultured human keratinocytes. Proc Natl Acad Sci USA 86:6367–6371, 1989
7. Schröder J, Christophers E: Identification of C5a des arg and an anionic neutrophil activating peptide (ANAP) in psoriatic scales. J Invest Dermatol 87:53–58, 1986
8. Camp RDR, Fincham NJ, Cunningham FM, Greaves MW, Morris J, Chu A: Psoriatic skin lesions contain biologically active amounts of an interleukin-1-like compound. J Immunol 137:3469–3474, 1986
9. Fincham NJ, Camp RDR, Gearing AJH, Bird CR, Cunningham FM: Neutrophil chemoattractant and IL-1 activity in samples from psoriatic skin lesions. J Immunol 140:4294–4299, 1988
10. Larsen CG, Anderson AO, Oppenheim JJ, Matsushima K: Production of interleukin 8 by human dermal fibroblasts and keratinocytes in response to interleukin 1 or tumor necrosis factor. Immunology 68:31–36, 1989
11. Mielke V, Bauman JGJ, Sticherling M, Ibs T, Zomershoe AG, Seligmann K, Hennicke H-H, Schröder J-M, Sterry W, Christophers E: Detection of neutrophil-activating peptide NAP/IL-8 and NAP/IL-8 mRNA in human recombinant IL-1 α and human recombinant tumor necrosis factor-α-stimulated human dermal fibroblasts. J Immunol 144:153–161, 1990
12. Schröder J-M, Mrowietz U, Christophers E: Purification and partial biologic characterization of a human lymphocyte-derived peptide with potent neutrophil-stimulating activity. J Immunol 140:3534–3540, 1988
13. Strieter RM, Shan SH, Showell HJ, Remick DG, Phan SH, Ward PA, Marks RM: Endothelial cell gene expression of a neutrophil chemotactic factor by TNF-a, LPS, and IL-1b. Science 243:1467–1469, 1989
14. Matsushima K, Oppenheim JJ: Interleukin 8 and MCAF: Novel inflammatory cytokines inducible by IL-1 and TNF. Cytokine 1:2–13, 1989
15. Dustin ML, Rothlein R, Bhan AK, Dinarello CA, Springer TA: Induction by IL-1 and interferon-gamma: tissue distribution, biochemistry, and function of a natural adherence molecule (ICAM-1). J Immunol 137:245–254, 1986
16. Bevilacqua MP, Stengelin S, Gimbrone MA Jr, Seed B: Endothelial leukocyte adhesion molecule 1: An inducible receptor for neutrophils related to complement regulatory proteins and lectins. Science 243:1160–1165, 1989
17. Gillitzer R, Pilarski L: In situ localization of CD45 isoforms in the human thymus indicates a medullary location for the thymic generative lineage. J Immunol 144:66–74, 1990
18. Gillitzer R, Berger R, Moll H: A reliable method for simulataneous demonstration of two antigens using a novel combination of immunogold-silver staining and immunoenzymatic labeling. J Histochem Cytochem 38:307–313, 1990
19. Pennica D, Nedwin GE, Hayflick JS, Seeburg PH, Derynck R, Palladino MA, Kohr WJ, Aggarwal BB, Goeddel DV: Human tumor necrosis factor: precursor structure, expression and homology to lymphotoxin. Nature 312:724–729, 1984
20. Hirano T, Yasukawa K, Koyama K, Iwamatu A, Tsunasawa S, Sakiyama F, Matsui H, Takahara Y, Taniguchi T, Kishimoto T: Complementary DNA for a novel human interleukin (BSF-2) that induces B lymphocytes to produce immunoglobulin. Nature 324: 73–74, 1986
21. Schmid J, Weissmann C: Induction of mRNA for a serine protease and a beta-thromboglobulin-like protein in mitogen-stimulated human leukocytes. J Immunol 139:250–256, 1987
22. Mueller C, Gershenfeld HK, Lobe CG, Okada CY, Bleackley RC, Weissman IL: A high proportion of T lymphocytes that infiltrate H-2-incompatible heart allografts in vivo express genes encoding cytotoxic cell-specific serine proteases, but do not express the MEL-14–defined lymph node homing receptor. J Exp Med 167:1124–1136, 1988
23. Greil R, Fasching B, Huber H: In situ hybridization for the detection of low copy numbers of c-abl oncogene mRNA in lymphoma cells: technical approach and comparison with results with anti-oncoprotein antibodies. Lab Invest 60:574–582, 1989
24. Lawrence JB, Singer RH: Quantitative analysis of in situ hybridization methods for the detection of actin gene expression. Nucleic Acids Res 13:1777–1799, 1985
25. Angerer LM, Dolecki GJ, Gagnon ML, Lum R, Wang G, Yang Q, Humphreys T, Angerer RC: Progressively restricted expression of a homeo box gene within the aboral ectoderm of developing sea urchin embryos. Genes Dev 3:370–383, 1989
26. Kragballe K, Fisher GJ, Voorhees JJ: Eicosanoids in inflammatory and immunologic skin disorders. In: Bos JD (ed.). Skin Immune System (SIS). CRC Press, Inc., Boca Raton, FL, 1989, pp 244–255
27. Schröder JM, Sticherling M, Henneicke HH, Preissner WC, Christophers E: IL-1α or TNF-α stimulate release of three NAP-1/IL-8-related neutrophil chemotactic proteins in human dermal fibroblasts. J Immunol 144:2223–2232, 1990
28. Barker JNWN, Sarma V, Mitra RS, Dixit VM, Nickoloff BJ: Marked synergism between tumor necrosis factor-alpha and interferon-gamma in regulation of keratinocyte derived adhesion molecules and chemotactic factors. J Clin Invest 85:605–608, 1990
29. Barker JNWN, Griffiths CEM, Mitra RS, Elder JT, Dixit V, Kunkel S, Nickoloff BJ: Keratinocyte-derived interleukin-8 (IL-8): regula-

tion by TPA and Urushiol and detection in inflamed skin (abstr). J Invest Dermatol 95:461, 1990

30. Griffiths CEM, Voorhees JJ, Nickoloff BJ: Characterization of intercellular adhesion molecule-1 and HLA-DR expression in normal and inflamed skin: modulation by recombinant gamma interferon and tumor necrosis factor. J Am Acad Dermatol 20:617–629, 1989

31. Reusch M, Studtmann K, Schröder MJ, Sticherling M, Christophers E: NAP-1/IL-8 is a potent mitogen for human keratinocytes in vitro (abstr). J Invest Dermatol 95:485, 1990

32. Turbitt ML, RJ Akhurst, SI White, RM Mackie: Localization of elevated transforming growth factor-alpha in psoriatic epidermis. J Invest Dermatol 95:229–232, 1990

33. Van Erp PEJ, De Mare S, Rijzzewijk JJ, Van de Kerkhof PCM: A sequential double immunoenzymatic staining procedure to obtain cell kinetic information in normal and hyperproliferative epidermis. Histochem J 21:343–347, 1989

34. Miyagawa S, Okada N, Takasaki Y, Iida T, Kitano Y, Yoshikawa K, Sakamoto K, Steinberg ML: Expression of proliferating cell nuclear antigen/cyclin in human keratinocytes. J Invest Dermatol 93:678–681, 1989

35. Bauer FW, Boezeman JBM, Engelen L v, de Grood RM, Ramaekers FCS: Monoclonal antibodies for epidermal population analysis. J Invest Dermatol 87:72–75, 1986

36. Prens EP, Benne K, van Damme J, Bakkus M, Brakel K, Benner R: Interleukin-1 and interleukin-6 in psoriasis. J Invest Dermatol 95:121S–124S, 1990

37. Sticherling M, Bornscheuer E, Schröder J-M, Christophers E: Localization of neutrophil-activating peptide-1/interleukin-8-immunoreactivity in normal and psoriatic skin. J Invest Dermatol 96:26–30, 1991

38. Larsen CG, Anderson AO, Appella E, Oppenheim JJ, Matsushima K: The neutrophil-activating protein (NAP-1) is also chemotactic for T-lymphocytes. Science 243:1464–1466, 1989